Applicant: **Rutgers, the State University et al.**
Title of Invention: **Extracts of Orange Peel for Prevention and Treatment of Cancer**

Attorney Ref.: **RU-0103**

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Date of Deposit **20 September 2000**

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By Suzanne Sparkman
Typed Name: **Suzanne Sparkman**

To the United States Receiving Office (RO/US):

Accompanying this transmittal letter is the above-identified International Application, including a completed Request form (PCT/RO/101). Please process the application according to the provisions of the Patent Cooperation Treaty.

The following requests are made of the RO/US:

1. **X** Preparation and Transmittal of Certified Copies of Priority Documents - Please prepare and transmit to the International Bureau a certified copy of the United States origin priority documents identified in Box VI of the Request form (37 CFR 1.451). To cover the cost of copy preparation and certification, the appropriate fee is indicated on the Fee Calculation Sheet (PCT/RO/101 Annex).

2. **X** Choice of International Searching Authority - It is requested that the International Search be performed by the following International Searching Authority. The appropriate Search fee for the below-named Authority is indicated on the Fee Calculation Sheet (PCT/RO/101 Annex).

X United States Patent and Trademark Office (ISA/US)
___ European Patent Office (ISA/EP)

3. ___ Supplemental Search Fees - Please charge any Supplemental Search fees that may be required by the United States International Searching Authority (ISA/US) to deposit account number 12-1086.

4. **X** Disclosure Information - In order to assist in screening the accompanying International Application for purposes of determining whether a license for foreign transmittal should and could be granted and for other purposes, the following information is supplied.

A. ___ There is no prior application relating to this invention.
B. **X** There is a prior application, US serial number **60/155,018** filed on **21 September 1999**. The prior application contain subject matter that is less than that of the International Application. The additional subject matter appears throughout the International Application.

5. **X** Request for Foreign Transmittal License - According to the provisions of 35 U.S.C. 184 and 37 CFR 5.11, a license to transmit the accompanying International Application to foreign agencies or international authorities is hereby requested.

Respectfully submitted,

Jane Massey Licata
Registration No. 32,257
Attorney/Agent for Applicant

Law Offices of Jane Massey Licata
66 E. Main Street
Marlton, New Jersey 08053
(856) 810-1515

IN[redacted]E PATENT COOPERATION TRE[redacted]
Before the International Bureau of WIPO

Applicant: Rutgers, the State University et al.

International Application No.: PCT/US00/25733

International Filing Date: 20 September 2000

Attorney Ref.: RU-0103

= *VIA FACSIMILE* =

International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Statement Under Article 19(1)

Dear Sirs:

Claims 1, 3-6 have been amended. Claim 2 has been deleted. Claims 7-11 are new. No new matter has been added by these amendments.

Respectfully submitted,

Jane Massey Licata
Registration No. 32,257

Date: 26 February 2001

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that the reference to claim 2 has been deleted since as filed claim 2 has been deleted. Claim 6 as filed corresponds to new claim 4 with the exception that the dependency has been corrected in light of the renumbering of the claims. Claim 7 as filed corresponds to new claim 5 with the exception that the reference to deleted claim 2 was removed. Claim 8 as filed corresponds to new claim 6 with the exception that the dependency has been amended in light of the claim renumbering.

Claims 7-9 were added to specify that the composition of the present invention can be a nutraceutical for prevention and treatment of cancer as taught in the specification as filed at page 9. No new matter has been added by this addition to the claims.

Claims 10 and 11 were added to specify that the composition of the present invention can be a dietary supplement for prevention and treatment of cancer as taught in the specification as filed at page 11. No new matter has been added by this addition to the claims.

Respectfully submitted,

Jane Massey Licata
Registration No. 32,257

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Enclosure - substitute pages 15 and 16

IN[redacted]E PATENT COOPERATION TRE[redacted]
Before the International Bureau of WIPO

Applicant: **Rutgers, the State University et al.**

International Application No.: **PCT/US00/25733**

International Filing Date: **20 September 2000**

Attorney Ref.: **RU-0103**

= VIA FACSIMILE =

International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Response to International Search Report

Dear Sirs:

This is in response to the International Search Report mailed 27 December 2000 setting a two (2) month period for reply.

Claim 1 was amended to include the language of using three or more polymethoxylated flavones as listed in response to the Search Report. Support for this amendment to the claim can be found in the specification as filed at page 4, where the components as listed in claim 1 are taught, at pages 4-6 where the activity of an extract containing multiple polymethoxylated flavones in taught, and at page 9, where a combination of polymethoxylated flavones is taught. No new matter has been added by this amendment to the claims.

Claim 2 as filed has been deleted as the subject matter of this claim was incorporated into claim 1. Claim 3 as filed is now claim 2 in the replacement claim set. Claim 4 as filed has been deleted in accordance with the deletion of as filed claim 2. Claim 5 as filed corresponds to new claim 3 with the exception

What is claimed is:

1. A composition comprising an extract of orange peel containing three or more polymethoxylated flavones, wherein said flavones are selected from the group consisting of 4',5,6,7,8-pentamethoxyflavone, 3',4',5,6,7,8-hexamethoxyflavone, 5, 6, 7, 3', 4'-pentamethoxyflavone, 5-hydroxy-6,7,8,3',4'-pentamethoxyflavone, 5-hydroxy-7,8,3',4'-methoxyflavone, 5,7-hydroxy-6,8,3',4'-methoxyflavone, 5,7,8,3',4'-pentamethoxyflavone, 5,7,8,4'-methoxyflavone, 3,5,6,7,8,3',4'-methoxyflavone, 5-hydroxy-3,6,7,8,3',4'-methoxyflavone, 5-hydroxy-6,7,8,4'-methoxyflavone, 5,6,7,4'-methoxyflavone, 7-hydroxy-3,5,6,8,3',4'-methoxyflavone, and 7-hydroxy-3,5,6,3',4'-methoxyflavone, and a physiologically acceptable carrier or excipient.

2. A composition comprising the extract of claim 1 and at least one other compound selected from the group consisting of rosemary extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a black tea extract, and a hydroxylated or methoxylated resveratrol analog.

3. A method for inhibiting tumor cell growth in an animal comprising administering to an animal the extract of claim 1.

4. A method for inhibiting tumor cell growth in an animal comprising administering to an animal the composition of claim 2.

5. A method for preventing or treating cancer in an animal comprising administering to an animal an effective amount of an extract of claim 1.

6. The method of claim 5 further comprising administering at least one additional compound selected from the group consisting of a rosemary extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a hydroxylated resveratrol analog, a black tea extract, and a methoxylated resveratrol analog.

7. A nutraceutical for prevention or treatment of cancer comprising the composition of claim 1 or 2.

8. The nutraceutical of claim 7 wherein said nutraceutical is administered orally as a tablet, capsule or liquid.

9. The nutraceutical of claim 7 wherein said nutraceutical is formulated for administration by inhalation, by injection, by rectally, or vaginally.

10. A dietary supplement for prevention or treatment of cancer comprising the composition of claim 1 or 2.

11. The dietary supplement of claim 10 wherein said dietary supplement is administered orally as a tablet, capsule or liquid.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
Before the United States International Preliminary
Examining Authority for the Patent Cooperation Treaty

Applicants:	Rutgers, the State University, et al.
International Application No.:	PCT/US00/25733
International Filing Date:	20 September 2000
Attorney Docket Number:	RU-0103

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I hereby certify that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Box PCT, Washington, D.C. 20231.

By _____
Typed Name: Jane Massey Licata, Registration No. 32,257

Assistant Commissioner for Patents
Box PCT
Washington, D.C. 20231

Dear Sir:

RESPONSE TO WRITTEN OPINION

This is in reply to the Written Opinion mailed August 29, 2001, setting a two (2) month period for response. Also, attached herewith is a copy of a replacement claim set. This claim set is identical to that originally provided to the International Preliminary Examining Authority in a paper dated February 26, 2001 except that an inadvertent error in claim 5 has been corrected i.e. this claim now correctly refers to a "composition". Applicants note that the application as published on March 29, 2201, did not include this replacement

claim set. Accordingly, Applicants request that the claims be replaced by the replacement claim set provided herewith.

Claim 1 has been suggested to lack novelty under PCT Article 33(2) as being anticipated by Nagy et al. (1979). Applicants have amended claim 1 to include the language of using three or more polymethoxylated flavones. Support for this amendment to the claim can be found in the specification as filed at page 4, where the components as listed in claim 1 are taught, at pages 4-6 where the activity of an extract containing multiple polymethoxylated flavones is taught, and at page 9 where a combination of polymethoxylated flavones is taught. No new matter was added by this amendment to the claim.

The Examiner suggests that this reference teaches the claimed compounds being obtained from citrus peel and methods to obtain the claimed compounds. Nagy et al. (1979) is a book chapter that discusses flavonoid constituents of citrus. Careful review of the chapter indicates that it does not teach extracting only orange peel to obtain flavenoid compounds. In addition, although only certain compounds are mentioned in this reference, such as tangeretin and sinensetin, the reference fails to teach the use of three or more flavones in combination as now claimed. Accordingly, this reference cannot anticipate the invention as now claimed.

Claims 1-6 have been suggested to lack novelty under PCT Article 33(2) as being anticipated by Peirce (1999). The Examiner suggests that this reference discloses that rosemary extract helps to fight cancer and significantly inhibited development of breast cancer. Applicants disagree with the Examiner's conclusions.

As mentioned above, claim 1, and by dependency claims 2-6, have been amended to refer to a composition containing three or more specific polymethoxylated flavones in combination, and the use of this composition either alone or with other herbal extracts to prevent or treat cancer.

The reference of Peirce (1999) is a book excerpt which teaches use of rosemary for curative properties. However, nowhere does this reference teach or suggest any compound extracted from orange peel, including none of the polymethoxylated flavones recited. Accordingly, this reference does not anticipate the present invention.

Claims 1-11 have been said to lack novelty under PCT Article 33(2) as being anticipated by Madis Botanicals. The Examiner suggests that this reference discloses that resveratrol prevents carcinogenesis, leukemia, and preneoplastic lesions or tumorigenesis.

The reference cited as Madis Botanicals is a package insert-type excerpt that lists the nutraceutical profile of resveratrol. Review of the reference reveals that it teaches

that Huzhang is a concentrated source of resveratrol. Nowhere does this reference teach or suggest any compounds extracted from oranges or orange peel specifically. Further, although the reference teaches use of resveratrol in cancer, it does not teach use of any of the compounds extracted from orange peel. Therefore, this reference does not anticipate the claims as amended which refer to use of three or more specific polymethoxylated flavone compounds extracted from orange peel in combination with other compounds such as resveratrol.

Claims 1-3 and 5 have been suggested to lack novelty under PCT Article 33(2) as being anticipated by Castleman. The Examiner suggests that this reference discloses that black tea has antioxidants and may be useful in cancer prevention.

The reference of Castleman is an excerpt from a book. The reference teaches use of tea to aid in the prevention of cancer. Nowhere does this reference teach or suggest any compounds extracted from oranges or orange peel specifically, as claimed in the amended claims, or their uses to treat cancer. Therefore, this reference cannot anticipate the claims as amended.

Claims 1-11 have been suggested to lack an inventive step under PCT Article 33(3) as being obvious over Nagy et al., in view of Peirce, Madis Botanicals, Castleman, Thomas, and Bailey. The Examiner suggests that these references combined teach use of plant extracts for treating or

preventing cancer and that Thomas et al. specifically teaches that carotenoid pigments from orange peels prevent cancer.

As discussed above, the claims have been amended to recite that certain polymethoxylated flavones are being used as a composition either as a combination of three or more compounds, or these three or more compounds in combination further with other plant extracts. These compositions are then claimed for use in treating or preventing cancer.

Also as discussed above, the references of Nagy et al., Peirce, Madis Botanicals, and Castleman fail to teach or suggest use of three or more polymethoxylated flavones as listed, extracted from orange peel, in any way, including prevention and treatment of cancer. Thomas (US Patent No. 5,830,738) discloses extraction of carotenoids from plants. Review of the patent, however, reveals that it teaches only carotenoids, not flavonoids, which are chemically distinct compounds. Therefore, this reference does not teach the present invention. The reference of Bailey (US Patent 5,859,293) discloses a process of extracting carnasic acid from rosemary and sage. Again, review of the patent, reveals that it teaches only carnasic acid from plants, not the compounds of the present invention extracted from orange peel, which are chemically distinct from carnasic acid.

Accordingly, this combination of references fails to make the invention obvious.

Respectfully submitted,

Jane Massey Licata

Jane Massey Licata
Registration No. 32,257

Date: October 26, 2001

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What is claimed is:

1. A composition comprising an extract of orange peel containing three or more polymethoxylated flavones, wherein said flavones are selected from the group consisting of 4',5,6,7,8-pentamethoxyflavone, 3',4',5,6,7,8-hexamethoxyflavone, 5, 6, 7, 3', 4'-pentamethoxyflavone, 5-hydroxy-6,7,8,3',4'-pentamethoxyflavone, 5-hydroxy-7,8,3',4'-methoxyflavone, 5,7-hydroxy-6,8,3',4'-methoxyflavone, 5,7,8,3',4'-pentamethoxyflavone, 5,7,8,4'-methoxyflavone, 3,5,6,7,8,3',4'-methoxyflavone, 5-hydroxy-3,6,7,8,3',4'-methoxyflavone, 5-hydroxy-6,7,8,4'-methoxyflavone, 5,6,7,4'-methoxyflavone, 7-hydroxy-3,5,6,8,3',4'-methoxyflavone, and 7-hydroxy-3,5,6,3',4'-methoxyflavone, and a physiologically acceptable carrier or excipient.

2. A composition comprising the extract of claim 1 and at least one other compound selected from the group consisting of rosemary extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a black tea extract, and a hydroxylated or methoxylated resveratrol analog.

3. A method for inhibiting tumor cell growth in an animal comprising administering to an animal the extract of claim 1.

4. A method for inhibiting tumor cell growth in an animal comprising administering to an animal the composition of claim 2.

5. A method for preventing or treating cancer in an animal comprising administering to an animal an effective amount of the composition of claim 1.

6. The method of claim 5 further comprising administering at least one additional compound selected from the group consisting of a rosemary extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a hydroxylated resveratrol analog, a black tea extract, and a methoxylated resveratrol analog.

7. A nutraceutical for prevention or treatment of cancer comprising the composition of claim 1 or 2.

8. The nutraceutical of claim 7 wherein said nutraceutical is administered orally as a tablet, capsule or liquid.

9. The nutraceutical of claim 7 wherein said nutraceutical is formulated for administration by inhalation, by injection, by rectally, or vaginally.

10. A dietary supplement for prevention or treatment of cancer comprising the composition of claim 1 or 2.

11. The dietary supplement of claim 10 wherein said dietary supplement is administered orally as a tablet, capsule or liquid.

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 27 August 2001 (27.08.01)	
International application No. PCT/US00/25733	**Applicant's or agent's file reference** RU-0103
International filing date (day/month/year) 20 September 2000 (20.09.00)	**Priority date** (day/month/year) 21 September 1999 (21.09.99)
Applicant GHAI, Geeta et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

20 April 2001 (20.04.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
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Authorized officer

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/21137 A1

(54) Title: EXTRACTS OF ORANGE PEEL FOR PREVENTION AND TREATMENT OF CANCER

(57) Abstract: Compositions and methods of inhibiting tumor cell growth and treating and preventing cancer are provided based on administration of an orange peel extract either alone or in combination with other phytochemicals.

EXTRACTS OF ORANGE PEEL FOR PREVENTION AND TREATMENT OF CANCER

Background of the Invention

Naturally occurring non-nutritive agents present in plants such as flavonoids, phenolic compounds, glucosinulates, terpenes and many others are believed to have disease preventive properties. Diets containing some of these substances have been shown to be protective against diseases such as colon and breast cancer in animals (Kuo, S.M. 1997. *Clin. Rev. Oncogenesis* 8:47-69; Verhoeven et al. 1996. *Cancer Epid. Biomark. Prev.* 5:733-748; Bradlow et al. 1991. *Carcinogenesis* 12:1571-1574; Lamartiniere et al. 1995. *Proc. Soc. Exp. Biol. Med.* 208:120-123). The clinical relevance of such natural phytochemicals is dependent on extrapolation from epidemiological data and from experiments in animal models of diseases of interest.

Purified flavenoid compounds isolated from citrus juice have been tested individually for their effects on carcinogenesis, tumor cell growth and invasion of tumor cells into normal cells (Attaway, J.A. 1994. In: *Food Phytochemicals for Cancer Prevention*, ACS Symposia Series #546, Huang et al. Eds., pp. 240-248). In particular the polymethyoxylated flavenoids, tangeretin and nobeletin, were shown to have anti-carcinogenic activity.

Extracts of bitter-orange peel are used as an herbal drug (Bisset, N.G. 1994. *Herbal Drugs and Phytopharmaceuticals*, CRC Press: Boca Raton). Conditions treated include loss of appetite and dyspeptic complaints. The main components of the extract include limonene and flavonoids such as neohesperidin and naringin.

Several patents disclose the use of various phytochemicals in combination with orange peel extract or

dried orange peel. CN 1200277 describes use of a composition composed of 16 plant components, one of which is dried orange peel, for treatment of psychosis and nervous system disease. CN 1116945 describes the use of orange peel along with several other natural products in a capsule form to sooth the liver, nourish the stomach, remove stasis, stop pain and cure various gastric diseases. CN 1111134 discloses an oral liquid containing orange peel, among other things, for treatment of neurastenia, chronic bronchitis, asthma, coronary heart disease, high blood lipid levels, hepatitis, cytopenia, senility and immune dysfunction. CN 1106673 is a patent for a disease-preventing nutrient tea that is produced from a variety of products, including soaked, crushed orange peel. CN 1077124 describes a Chinese herb preparation for treatment of iron-deficiency anemia that is composed of a number of ingredients, including dried orange peel. Finally, a Japanese patent (JP 57156761) discloses a heat-generating pad for orthopedic diseases that contains extracts and powders of many plants, including orange peel.

It has now been found that an extract of orange peel has biological activity as a treatment and preventative agent for cancer.

Summary of the Invention

An object of the present invention is an extract of orange peel which comprises 4',5,6,7,8-pentamethoxyflavone and 3',4',5,6,7,8-hexamethoxyflavone. The composition may further comprise other polymethoxylated flavones.

Another object of the present invention is a composition which comprises an extract of orange peel and rosemary extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a black tea extract, and/or a hydroxylated or methoxylated resveratrol analog.

Another object of the present invention is to provide a method for inhibiting tumor cell growth in an animal

comprising administering to an animal an orange peel extract which is administered alone or in combination with rosemary extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a black tea extract, and/or a hydroxylated or methoxylated resveratrol analog.

Another object of the present invention is to provide a method for preventing or treating cancer in an animal which comprises administering to an animal an effective amount of an orange peel extract which is administered alone or in combination with rosemary extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a black tea extract, and/or a hydroxylated or methoxylated resveratrol analog.

Detailed Description of the Invention

Unlike many phytochemicals, orange peel extract is lipid soluble, a property which is desirable in many drug products because passage across biological membranes, and ultimately bioavailability, is enhanced. Orange peel and its extracts have been used in a variety of herbal drug products in combination with many different plant components and extracts. However, none of the previous research on orange peel or its extracts has examined or demonstrated activity against tumor cell growth or cancer. It has now been shown that orange peel extract inhibits tumor growth *in vivo*.

Orange peel extract is a mixture of highly bioactive and organic soluble, methylated flavonoids. An extract was obtained from cold-pressed peel oil solids, a waste product from the orange juice industry. The peel oil solids were dissolved in warm ethanol and, after several repeated washes, became a standardized product, with a reproducible amount of flavonoids. The extract comprises a mixture of various analogs and homologs of methylated flavonoids.

Experiments were performed to isolate and identify components in the orange peel extract. Methylated flavonoids from the orange peel extract were analyzed by either reverse-

phase or normal-phase high performance liquid chromatography (HPLC). During normal phase HPLC the conditions included use of a silica gel HPLC column (MacMod Analytical Co., Chadds Ford, PA) of dimensions 4.6 mm i.d. x 25 cm length and a solvent gradient that started at 90% hexane and went to 90% chloroform in 20 minutes with a final hold at 90% chloroform for an additional 20 minutes. Separated components or peaks were then identified using HPLC coupled with mass spectrometry (HPLC-MS). Atmospheric pressure chemical ionization mass spectrometry was used for molecular weight determinations. HPLC-MS techniques such as particle beam (EI) introduction was used to produce standard fragmentation patterns of the methylated flavonoids. Standards for many of the compounds were obtained from the Florida Department of Citrus. Using these techniques the following components were identified: 5,6,7,3',4'-pentamethoxyflavone (also known as sinensetin), 5,6,7,8,3',4'-hexamethoxyflavone (also known as nobeletin), 5,6,7,8,4'-pentamethoxyflavone (also known as tangeretin), 5-hydroxy-6,7,8,3',4'-pentamethoxyflavone (also known as auranetin), 5-hydroxy-7,8,3',4'-methoxyflavone, 5,7-hydroxy-6,8,3',4'-methoxyflavone, 5,7,8,3',4'-pentamethoxyflavone, 5,7,8,4'-methoxyflavone, 3,5,6,7,8,3',4'-methoxyflavone, 5-hydroxy-3,6,7,8,3',4'-methoxyflavone, 5-hydroxy-6,7,8,4'-methoxyflavone, 5,6,7,4'-methoxyflavone, 7-hydroxy-3,5,6,8,3',4'-methoxyflavone, and 7-hydroxy-3,5,6,3',4'-methoxyflavone.

The *in vivo* tumor inhibitory effects of the complete (including all 14 identified compounds) orange peel extract was tested in an orthotransplant model (Telang, N.T. et al. 1990. *Cell Regulat.* 1:863-872). Mice were transplanted with oncogene-expressing, preneoplastic breast epithelial cells. Mice were then divided into groups with the control group fed AIN-76A diet alone. Another group of mice was fed AIN-76A diet supplemented with 5000 ppm orange peel extract. After 12 weeks of continuous feeding, all mice in the control group

exhibited palpable tumor formation at the transplant sites (100% tumor incidence). In contrast, the group fed diet supplemented with the orange peel extract had a 0% tumor incidence (0/5 mice). Weight gains in the groups were comparable indicating that the orange peel extract had little to no systemic toxicity.

The orange peel extract was then tested in an *in vivo* model for colon cancer. Female CF-1 mice were injected with azoxymethane (AOM) once a week for four weeks at increasing doses (5, 10, 10 and 10 mg/kg). Orange peel extract was administered in the diet (0.2%) starting two weeks before the first AOM injection, during and continuing until the end of the experiment at 24 weeks. At week 24, the mice were given one last dose of AOM (10 mg/kg). The mice were then sacrificed and their colons removed (from anus to caecum). The colons were opened longitudinally, rinsed with normal saline, and stapled to a plastic sheet. The colon samples were placed in a 10% neutral buffered formalin solution for 24 hours. The entire colon was stained with 0.2% methylene blue dissolved in phosphate buffered saline for 20 minutes. The whole mount of colon samples were then examined using light microscopy for the presence of aberrant crypt (AC) or aberrant crypt foci (ACF). Both ACF and AC are biomarkers for colon cancer. Cancer prevention diets have been shown to reduce formation of ACF and AC. Mice fed nordihydroxyguaiaretic acid (NDGA) in the diet (0.2%) were used as controls. The results are shown below in Table 1.

Table 1
Effect of Feeding Orange Peel Extract on AOM-Induced Formation of Aberrant Crypt Foci (ACF) in Mice

Lesion	Negative Control	Positive Control	0.2% NDGA	0.2% Orange Peel
ACF/colon	0	5.2±1.2	2.7±0.9	2.7±0.8

AC/colon	0	37±5.9	9.4±2.2	12.6±2.8
AC/ACF	0	7.1	3.5	4.7
ACF: 1 AC/colon	0	15.0±2.5	6.8±1.5	6.4±1.4
ACF: 2 AC/colon	0	5.5±1.2	1.0±0.3	2.0±0.3
ACF: 3 AC/colon	0	1.0±0.4	0.2±0.2	0.2±0.2
ACF: 4 AC/colon	0	1.0±0.4	0	0.2±0.2
ACF: 5 AC/colon	0	0.2±0.2	0	0
ACF: 6 AC/colon	0	0.3±0.3	0	0.2±0.23
ACF: 7 AC/colon	0	0.2±0.2	0	0

There was a 48% and 48% inhibition of the number of ACF per colon with NDGA and orange peel extract treatment, respectively. In addition, the ratio of AC/ACF was inhibited by 51% and 34%, with NDGA and orange peel extract treatment, respectively. These data demonstrate the efficacy of the orange peel extract in this animal model of colon cancer.

In a similar experiment in the mouse colon cancer model, CF-1 mice were injected with AOM (5, 10, 10 and 10 mg/kg) starting at 6 weeks of age, once each week and then once at 37 weeks after the first dose of AOM. Throughout the treatment period, mice received either an AIN 76A diet or test compound in AIN 76A diet at 2 weeks before the first dose of AOM and continuing until the end of the experiment. The test compounds were NDGA (0.2%) and orange peel extract (0.2%). Colon samples were again obtained at sacrifice, stored in 10%

formalin phosphate buffer, and then colon tumor number was determined. The results are shown in Table 2.

Table 2
Effect of Dietary Orange Peel Extract Treatment on AOM-Induced Colon Tumorigenesis in Mice

Treatment	Number of Animals	Body Weight (g)	Colon Tumors Per Mouse	Percent Incidence (%)
no AOM (negative control)	15	51.3±1.9	0	0
AOM	27	46.7±1.9	0.52±0.12	44
0.2% NDGA + AOM	11	45.8±2.1	0.27±0.14	27
0.2% Orange Peel + AOM	17	46.7±2.2	0.29±0.11	29

The data show that treatment with orange peel extract inhibited tumor development in AOM-treated mice to the same extent as the control comparison compound, NDGA, supporting the efficacy of orange peel extract as an anti-tumorigenic agent.

In addition to testing for the activity of the complete orange peel extract, two of the identified extract components, tangeretin and nobeletin, were tested for their combined activity in a cell proliferation assay. The growth of W138 (normal) and W138VA (transformed) cells was tested in the presence of a mixture of tangeretin and nobeletin. The dye crystal violet was used for measuring growth of the cells. Cells were treated with either tangeretin alone (0, 1, 5, 10, 20 or 50 μg/ml), nobeletin alone (0, 1, 5, 10, 20 or 50 μg/ml) or a mixture of the two compounds at a total concentration of the two flavenoids of 0, 1, 5, 10, 20 or 50 μg/ml. When used alone, tangeretin and nobeletin produced only marginal effects to inhibit cell growth in transformed cells, even at

the highest dose tested, and had no effect on normal cell growth. In contrast, when administered as a mixture, tangeretin and nobeletin showed synergistic activity, with growth inhibition produced in transformed cells, in a dose dependent manner. There was no appreciable effect of the mixture on normal cell growth. These data confirm the results of the experiment in whole animals where orange peel extract, containing tangeretin and noveletin, had anti-tumorigenic activity. Further, when an extract containing 30% of the methylated flavenoids, including tangeretin and nobeletin was tested in this same assay there were significant inhibitory effects of cell proliferation at doses of 20 and 50 μg/ml. The range of doses of the extract tested was 0, 1, 5, 10, 20 and 50 μg/ml. These data provide evidence for a synergistic effect of the polymethylated flavonoids in arresting and inhibiting the growth of tumor cells.

Experiments were also performed in a preclinical cell culture model for human ductal breast carcinoma in situ (DCIS). The human breast-derived preneoplastic cell line 184-B5/HER expressed HER-2/neu, p53 and EGFR but not ER, therefore resembling the clinical DCIS. Initial dose-response experiments compared the growth inhibitory effect of orange peel extract on the parental 184-B5 cells and the HER-2/neu oncogene-expressing 184-B5/HER cells. Relative to parental cells, orange peel extract was at least two times more effective as a growth inhibitor for 184-B5/HER cells. Orange peel extract at the maximum cytostatic dose of 100 ppm accumulated the cells in the G0/G1 phase and inhibited the S+G2/M phase of the cell cycle, leading to down-regulation of cell cycle progression. This alteration in the cell cycle progression resulted in a 5-fold increase in the G0/G1: S+G2/M ratio. Treatment of 184-B5/HER cells with 100 ppm orange peel extract resulted in a 47.5% decrease in immunoreactivity to phosphotyrosine (marker for tyrosine kinase activity) and a 157.7% increase in immunoreactivity to the cyclin dependent

kinase inhibitor $p16^{INK4}$. In addition, there was a selective induction of apoptosis in 184-B5/HER cells but not in parental 184-B5 cells. Treatment of 184-B5/HER cells with 100 ppm orange peel extract induced a 7.6-fold increase in sub G0/G1 (apoptotic) population. Consistent with the induction of apoptosis, immunoreactivity to anti-apoptotic Bcl-2 was decreased by 33%.

Based upon the experiments described herein, it is believed that compositions comprising orange peel extract or a combination of components of the orange peel extract including but not limited to tangeretin and nobeletin, may be included in foods and dietary supplements or "nutraceuticals" for prevention or treatment of cancer. One of skill can use the results of experiments in cells and animals described herein to determine effective amounts to be administered to other animals, including humans. By "effective amount" it is meant a concentration that inhibits tumor growth either *in vitro* in cells or *in vivo* in animals. For example, human test doses can be extrapolated from effective doses in cell studies, such as IC_{50} values, or from effective doses *in vivo* by extrapolating on a body weight or surface area basis. Such extrapolations are routine in the art. Compositions comprising orange peel extracts can be formulated for administration as a food supplement using one or more fillers. Alternatively, compositions comprising these extracts can be administered as conventional pharmaceuticals using one or more physiologically acceptable carriers or excipients. Nutraceutical compositions can be formulated for administration by any route including, but not limited to, inhalation or insufflation (through mouth or nose), oral, buccal, parenteral, vaginal, or rectal administration. In one embodiment, oral administration, the compositions are added directly to foods and ingested as part of a normal meal. Various methods are known to those skilled in the art for addition or incorporation of nutraceuticals into foods.

Compositions for use in the present invention can also be administered in the form or tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents, fillers, lubricants, disintegrants, or wetting agents. Examples of specific compounds for use in formulating tablets and capsules are described in detail in the U.S. Pharmacopeia. Tablets comprising the extract can also be coated by methods well known in the art. Liquid preparations for oral administration can also be used. Liquid preparations can be in the form of solutions, syrups or suspensions, or a dry product for reconstitution with water or another suitable vehicle before use. Such liquid preparations can be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents, emulsifying agents, non-aqueous vehicles, and preservatives. Again, specific additives are well known to those of skill and are listed in places such as the U.S. Pharmacopeia. In one embodiment, the oral preparation is formulated to provide controlled time release of the active nutraceutical components. For buccal administration the extract can be formulated as a tablet or lozenge.

For administration by inhalation, compositions for use in the present invention can be delivered in the form of an aerosol spray in a pressurized package or as a nebulizer, with use of suitable propellants. In the case of a pressurized aerosol, the dosage unit can be determined by providing a valve to deliver a metered dose.

Parenterally administered compositions are formulated to allow for injection, either as a bolus or as a continuous infusion. Formulations for injection can be prepared in unit dosage forms, such as ampules, or in multi-dose units, with added preservatives. The compositions for injection can be in the form of suspensions, solutions, or emulsions, in either oily or aqueous vehicles. They may also contain formulatory agents such as suspending agents, stabilizing agents, and/or

dispersing agents. The active ingredient may also be presented in powder form for reconstitution with a suitable vehicle before use. Specific examples of formulating agents for parenteral injection are found in the U.S. Pharmacopeia.

For rectal administration or vaginal administration, compositions for use in of the present invention can be formulated as suppositories, creams, gels, or retention enemas.

For dietary supplements, the extract can be added in concentrations up to 5% by weight and mixed according to methods routine in the art. Dietary supplements for animals can be prepared in a variety of forms including, but not limited to, liquid, powder, or solid pill forms. In the present invention, the orange peel extract can administered either alone or in combination with other phytochemicals known to affect tumor cell growth, where combining compounds or extracts would lead to synergistic effects. Examples of other phytochemicals which can be used in combination with orange peel extract include, but are not limited to, resveratrol and its hydroxylated and methoxylated analogs, rosemary extract, black tea extracts, Mexican Bamboo, and Huzhang extracts.

Many plants, such as Mexican Bamboo and Huzhang, contain high amounts of an active component known as resveratrol. Resveratrol is a well known, biologically active phytochemical. Resveratrol and its hydroxylated and methoxylated analogs have been shown to have activity both *in vitro* and *in vivo* to affect cell proliferation and tumor cell growth. Resveratrol and several of its analogs (3,5-dihydroxystilbene: R-1; 3, 3', 4, 5'-tetrahydroxystilbene: R-2; 3, 4, 4', 5-tetrahydroxystilbene: R-3; 3, 3', 5, 5'-tetrahydroxystilbene (R-4), 3, 3', 4, 5, 5'-pentahydroxystilbene: R-5; 3, 5-dimethoxystilbene: MR-1; 3, 4', 5-trimethoxystilbene: MR-0; 3, 3', 4, 5'-tetramethoxystilbene: MR-2; 3, 4, 4', 5-tetramethoxystilbene:

MR-3; 3, 3', 5' 5'-tetramethoxystilbene: MR-4; and 3, 3', 4, 5, 5'-pentamethoxystilbene: MR-5) were evaluated in cell culture studies using standard methodologies.

W138 human diploid fibroblasts and cancerous SV40-transformed W138 cells (W138VA) were used in a cell proliferation assay. Growth rate and viability of these cells was determined following addition of resveratrol or one of its analogs. Doses tested ranged from 50 ng to 300 μg per ml or 1 μM to 100 μM concentrations in culture media. Resveratrol inhibited cell growth at concentrations less than 10 μM. The resveratrol analogs R3 and MR-0 also inhibited cell growth. At a concentration of 1 μM, MR-3 completely blocked proliferation of W138VA cells, although it had no effect on growth of W138 cells. MR-4 inhibited growth of W138 cells but not W138VA cells at doses of 100 μM. MR-1 was not active as an inhibitor of cell growth even at doses as high as 100 μM.

Treatment of W138 and W138VA cells with resveratrol and its analogs also led to morphological changes in the cells. Treatment of W138 cells with resveratrol and its analogs R-1 and R-3 led to elongation of normal W138 cells. Methoxy analogs such as MR-0 and MR-3 caused the flattening of W138 cells. This flattening was accompanied by an increase in neutral β-galactosidase activity as indicated by an increase in staining. An increase in activity of β-galactosidase is characteristic of senescent cells, indicating that these analogs modulate the life-span of normal cells.

Resveratrol and its analogs were also tested in preneoplastic 184-B5/HER human mammary epithelial cells. Results showed that there was a dose-dependent inhibition of growth in response to treatment with resveratrol as well as the methoxy derivatives MR-0, MR-2 and MR-3. The concentration that inhibited growth by 50% (IC_{50}) for the tested compounds were: resveratrol, 10.5 μM; MR-0, 10.5 μM; MR-2 120 μM; MR-3, 1.0 μM. A cell cycle analysis revealed that treatment with MR-0, MR-2 and MR-3 resulted in

progressive arrest of cells in the G2/M phase relative to solvent-treated control cultures and that MR-3 was the most effective compound.

The *in vivo* tumor inhibitory effects of MR-3 were tested in an orthotransplant model. Mice were transplanted with oncogene-expressing, preneoplastic breast epithelial cells. Mice were then divided into groups with the control group fed AIN-76A diet alone. Another group of mice was fed AIN-76A diet supplemented with MR-3 (400 ppm). After 12 weeks of continuous feeding, all mice in the control group exhibited palpable tumor formation at the transplant sites (100% tumor incidence). In contrast, the group fed diet supplemented with the analog MR-3 had a 20% tumor incidence, with only one mouse of the five tested exhibiting tumor growth. Weight gains in the groups were comparable indicating that the analog had little toxicity.

This series of studies, both *in vitro* and *in vivo*, indicated that resveratrol as well as analogs of resveratrol have biological activity related to preventing progression of cancer in cells.

Extracts of rosemary have also been shown to have anti-tumor activity and chemopreventive properties (Huang et al. 1994. *Cancer Res.*54:701-708; Tokuda et al. 1986. *Cancer Lett.* 33:279-285; Singletary et al. 1996. *Cancer Lett.* 104:43-48; Singletary, K.W. and J.M. Nelshoppen. 1991. *Cancer Lett.* 60:169-175). For example, a diet containing 1% of rosemary extract significantly inhibited the initiation of mammary tumorigenesis in rats (Singletary, K.W. and J.M. Nelshoppen. 1991. *Cancer Lett.* 60:169-175). Palpable tumor incidence in rats fed the rosemary extract was 47% less than that of rats fed a control diet. Therefore, rosemary extracts were cancer preventive.

Black tea and its extracts have also been well-studied as potential pharmacological agents. Epidemiological studies have suggested that tea consumption has a protective effect

against certain forms of human cancer (Stoner, G.D. and H. Mukhtar. 1995. *J. Cell Biochem. Suppl.* 22:169-180; Fujiki et al. 1996. *Nutr. Rev.* 54:S67-S70). In addition, extracts of black tea in particular have been shown to be potent inhibitors of tumorigenesis in several animal model systems (Javed et al. *Biomed. Environ. Sci.* 11:307-313; Yang et al. 1997. *Carcinogenesis* 18:2361-2365; Weisberger et al. 1998. *Carcinogenesis* 19:229-232; Rogers et al. 1998. *Carcinogenesis* 19:1269-1273). Therefore, black tea extracts are known to be tumor preventive agents.

Accordingly, it is believed that a combination diet of dietary supplement comprising orange peel extract and at least one other phytochemical will also be useful to treat or prevent cancer in animals, including humans. Orange peel extract may be used in combination with rosemary extract, resveratrol and its analogs, Mexican Bamboo or Huzhang extracts, and black tea extracts. Doses of each extract used in the combination product are selected based on known activity of the extract in animals or cells.

What is claimed is:

1. An extract of orange peel comprising 4',5,6,7,8-pentamethoxyflavone and 3',4',5,6,7,8-hexamethoxyflavone.

2. The extract of claim 1 further comprising at least one compound selected from the group consisting of 5-hydroxy-6,7,8,3',4'-pentamethoxyflavone, 5-hydroxy-7,8,3',4'-methoxyflavone, 5,7-hydroxy-6,8,3',4'-methoxyflavone, 5,7,8,3',4'-pentamethoxyflavone, 5,7,8,4'-methoxyflavone, 3,5,6,7,8,3',4'-methoxyflavone, 5-hydroxy-3,6,7,8,3',4'-methoxyflavone, 5-hydroxy-6,7,8,4'-methoxyflavone, 5,6,7,4'-methoxyflavone, 7-hydroxy-3,5,6,8,3',4'-methoxyflavone, and 7-hydroxy-3,5,6,3',4'-methoxyflavone. .

3. A composition comprising the extract of claim 1 and at least one other compound selected from the group consisting of rosemary extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a black tea extract, and a hydroxylated or methoxylated resveratrol analog.

4. A composition comprising the extract of claim 2 and at least one other compound selected from the group consisting of rosemary extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a black tea extract, and a hydroxylated or methoxylated resveratrol analog.

5. A method for inhibiting tumor cell growth in an animal comprising administering to an animal the extract of claim 1 or claim 2.

6. A method for inhibiting tumor cell growth in an animal comprising administering to an animal the composition of claim 3 or claim 4.

7. A method for preventing or treating cancer in an animal comprising administering to an animal an effective amount of an extract of claim 1 or claim 2.

8. The method of claim 7 further comprising administering at least one additional compound selected from the group consisting of a rosemary extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a hydroxylated resveratrol analog, a black tea extract, and a methoxylated resveratrol analog.

What is claimed is:

1. An extract of orange peel comprising 4',5,6,7,8-pentamethoxyflavone and 3',4',5,6,7,8-hexamethoxyflavone.

2. The extract of claim 1 further comprising at least one compound selected from the group consisting of 5-hydroxy-6,7,8,3',4'-pentamethoxyflavone, 5-hydroxy-7,8,3',4'-methoxyflavone, 5,7-hydroxy-6,8,3',4'-methoxyflavone, 5,7,8,3',4'-pentamethoxyflavone, 5,7,8,4'-methoxyflavone, 3,5,6,7,8,3',4'-methoxyflavone, 5-hydroxy-3,6,7,8,3',4'-methoxyflavone, 5-hydroxy-6,7,8,4'-methoxyflavone, 5,6,7,4'-methoxyflavone, 7-hydroxy-3,5,6,8,3',4'-methoxyflavone, and 7-hydroxy-3,5,6,3',4'-methoxyflavone. .

3. A composition comprising the extract of claim 1 and at least one other compound selected from the group consisting of rosemary extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a black tea extract, and a hydroxylated or methoxylated resveratrol analog.

4. A composition comprising the extract of claim 2 and at least one other compound selected from the group consisting of rosemary extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a black tea extract, and a hydroxylated or methoxylated resveratrol analog.

5. A method for inhibiting tumor cell growth in an animal comprising administering to an animal the extract of claim 1 or claim 2.

6. A method for inhibiting tumor cell growth in an animal comprising administering to an animal the composition of claim 3 or claim 4.

7. A method for preventing or treating cancer in an animal comprising administering to an animal an effective amount of an extract of claim 1 or claim 2.

8. The method of claim 7 further comprising administering at least one additional compound selected from the group consisting of a rosemary extract, a Mexican Bamboo extract, a Huzhang extract, resveratrol, a hydroxylated resveratrol analog, a black tea extract, and a methoxylated resveratrol analog.

INTERNATIONAL SEARCH REPORT

Internat[redacted] application No.
PCT/US00/25733

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 6/00, 7/00, 7/ 42, 7/44, 37/05, 37/22
US CL : 424/59.60, 63, 69, 195.1, 400,,401, 448; 426/425, 428; 435/ 209, 267; 514/733,736, 844, 846,847, 887

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/59.60, 63, 69, 195.1, 400,,401, 448; 426/425, 428; 435/ 209, 267; 514/733,736, 844, 846,847, 887

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Please See Continuation Sheet

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X – Y	NAGY, S. et al. Citrus Science and Technology.Westport:AVI. 1977, Vol. 1, page 415, lines 40-42; page 416, lines 15-41, pages 415-419.	1,2 ----- 3-8
X	PEIRCE, Andrea. Practical Guide to Natural Medicines. New York, William Morrow and Company. 1999, pages 551-554, especially page 553. lines 5-7.	1,3,5,7
X	Madis Botanicals, Inc. ResveraPure™ Resveratrol PE 8%.Lines 6-7 and 15-31.	1-2,3,5,7,8
X	CASTLEMAN. Michael. The Healing Herbs. Emmaus: Rodale Press. 1991, pages 348-350, especially page 349, column 2, lines 5-10.	1,3,5,7
Y	US 5, 830, 738 A (THOMAS et al.) 03 November 1998, column 1,lines 22-62.	1-4
Y	US 5,859, 293 A (BAILEY et al.) 12 January 1999, (12.01.1999), column 1, lines 29-34; column 2, lines 10-15.	3,4,6-8

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search November 6, 2000	Date of mailing of the international search report 27 DEC 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer Kailash C. Srivastava Telephone No. (703)-308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

Continuation of B. FIELDS SEARCHED Item 2: PEIRCE, A. (Ed.) Practical Guide to Natural Medicines. New York. Wiliam Morrow and Company, Inc.,1999, Pages 551-554.
NAGY, S., SHAW, P.E., VELDHUIS, M.K. (Eds.) Citrus Science and Tcehnology. Westport: AVI Publishing, Co., Inc., 1979, Vol.1, pages 415-419; Page 415, Lines 40-42; Page 416, Lines15-41.
CASTLEMAN, M. The Healing Herrbs. Emmaus: Rodale Press,1991, Pages 348-350, especially page 349,Column 2, lines5-10

Continuation of B. FIELDS SEARCHED Item 3: CAS, USPT, JPAB, EPAB, DWPIorange peel extarct, japanese knotwood, Polygonum cuspidatum, huzhang, mexican bamboo, hydroxyflavone, hexamethoxyflavone, rosemary, blacktea, hazhang extract, resveratrol analog, cancer treatment, tumor prevention,sinensetin, nobeeltin, tangeretin, auranetin)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

8

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference RU-0103	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No PCT/US00/25733	International filing date (day/month/year) 20 September 2000 (20.09.2000)	Priority date (day/month/year) 21 September 1999 (21.09.1999)

International Patent Classification (IPC) or national classification and IPC

IPC(7): A61K 6/00, A61K 7/00, A61K 7/42, A61K 7/44, A61K 37/05, A61K 37/22 and US Cl.: 424/59.60, 63, 69, 195.1, 400, 401, 448, 426/425, 428; 435/209, 267; 514/733,736, 844, 846,847, 887

Applicant

THE STATE UNIVERSITY, RUTGERS

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets, including this cover sheet.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I [X] Basis of the report
   - II [ ] Priority
   - III [ ] Non-establishment of report with regard to novelty, inventive step and industrial applicability
   - IV [ ] Lack of unity of invention
   - V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI [ ] Certain documents cited
   - VII [ ] Certain defects in the international application
   - VIII [ ] Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20 April 2001 (20.04.2001)	20 January 2002 (20.01.2002)
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703)305-3230	Authorized officer DR. Kailash C. Srivastava Telephone No. (703)-308-0196

Form PCT/IPEA/409 (cover sheet) (July 1998)

INTERNATIONAL PRELIMI[redacted] EXAMINATION REPORT

International [redacted]tion No
PCT US00 25733

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed.

☒ the description:
pages 1-14 as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

☒ the claims:
pages NONE , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages NONE , filed with the demand
pages 15 and 16 , filed with the letter of 26 October 2001 (26.10.2001)

☒ the drawings:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

☒ the sequence listing part of the description:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination(under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. ☒ The amendments have resulted in the cancellation of:

☒ the description, pages NONE

☒ the claims, Nos. NONE

☒ the drawings, sheets: ~~fig~~ NONE

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims 1-11	YES
	Claims NONE	NO
Inventive Step (IS)	Claims NONE	YES
	Claims 1-11	NO
Industrial Applicability (IA)	Claims 1-11	YES
	Claims NONE	NO

2. CITATIONS AND EXPLANATIONS

Claims 1-11 lack an inventive step under PCT artucle 33 (3) as being obvious over Nagy et al., in view of Peirce, Madis Botanicals, Casleman, Thomas and Bailey. Nagy et al., disclose the claimed compounds being obtained from the citrus peel and methods to obtain the referred compounds. Nagy et al. do not disclose the anticarcinogenic or tumor inhibition propeties of orange peel or other plant species recited in claims 2-11. These authors also do not disclose the neutraceutical or dietary supplements containing orange peel, with or without other plant extracts claimed in Claims 2 and 6) for inhibiting tumor development or prevention of cancer. Thomas et al., however, disclose that carotenoid pigments obtained from orange peels and other plants prevent cancer upon ingestion of these chemicals. Peirce discloses that Rosemary extract helps fight cancer and has been shown to significantly inhibit development of breast cancer. Madis Botanicals discloses that resveratrol (present in Huzhang or Mexican bamboo) prevents carcinogenesis, promyelocytic leukemia and preneoplastic lesions or tumerogenesis. Casdeman discloses that black tea has antioxidants and therefore it may also be helpful in cancer prvention. Similarly, Bailey et al., and Peirce disclose prevention, inhibition or delayed onset of certian types of cancers when extracts from Rosemary and other plants are ingested. Castelman discloses prevention of cancer by black tea and Madis Botanicals discloses neutraceutical preparations of resveratrol obtained from Huzhang or knotweed. It is also known that Huzhang or knotweed or Mexican bamboo or giant knotwood are all Polygamum cuspidatum and resveratrol is an antioxidant obtained from this plant species. All the references cited also disclsoe that the plant extracts cited herein are comprised of antioxidants and it is the antioxidant component of these plants that is effective in either inhibiting or preventing, or late onset of different types of cancer. Thus, it was known in the prior art that these plant species or extract therefrom are applicable in inhibition and or prevention of some types of cancer. Also known in the art are the neutraceutical and dietary supplements of these plant extracts. In view of the fact that applicants' invention is also on prevention or inhibition of cancer with neutraceutical or dietary supplements of the said plant extracts, applicants' invention is obvious over the teachings of prior art and therefore, is neither novel, nor has an inventive step.

---------- NEW CITATIONS ----------

NONE.

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For Receiving Office use only

International Application No.

International Filing Date 10/088664

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* RU-0103

Box No. I TITLE OF INVENTION

EXTRACTS OF ORANGE PEEL FOR PREVENTION AND TREATMENT OF CANCER

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

RUTGERS, THE STATE UNIVERSITY
Old Queens Building
Somerset and George Streets
New Brunswick, New Jersey 08901 US

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

GHAI, Geeta
250 Gallinson Drive
Murray Hill, NJ 07974 us

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

LICATA, Jane Massey; TYRRELL, Kathleen A.
Law Offices of Jane Massey Licata
66 E. Main Street
Marlton, New Jersey 08053 US

Telephone No. 856-810-1515

Facsimile No. 856-810-1454

Teleprinter No.

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet is not to be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

ROSEN, Robert T.
347 Harrier Drive
Monroe Township, NJ 08831 US

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

HO, Chi-Tang
32 Jernee Drive
East Brunswick, NJ 08816 US

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

CHEN, Kuang Yu
4 Silverthron Lane
Belle Mead, NJ 08502 US

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: CN

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

TELANG, Nitin
788 Colonial Avenue
Pelham Manor, NY 10803 US

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: IN

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet is not to be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

LIPKIN, Martin
535 East 86th Street
New York, New York 10028 US

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

HUANG, Mou Tuan
266 Alfred Street
Englewood Cliffs, NJ 07632 US

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

BOYD, Charles
3330 Paty Drive
Honolulu, Hawaii 96822

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

CSISZAR, Katalin
3330 Paty Drive
Honolulu, Hawaii 96822 US

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: HU

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

☒ AP **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, MZ Mozambique, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

☒ EA **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ EP **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ OA **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

☒ AE United Arab Emirates
☒ AG Antigua and Barbuda
☒ AL Albania .....
☒ AM Armenia .....
☒ AT Austria .....
☒ AU Australia .....
☒ AZ Azerbaijan
☒ BA Bosnia and Herzegovina .....
☒ BB Barbados
☒ BG Bulgaria .....
☒ BR Brazil .....
☒ BY Belarus .....
☒ BZ Belize
☒ CA Canada
☒ CH and LI Switzerland and Liechtenstein
☒ CN China .....
☒ CR Costa Rica .....
☒ CU Cuba .....
☒ CZ Czech Republic .....
☒ DE Germany .....
☒ DK Denmark .....
☒ DM Dominica
☒ DZ Algeria .....
☒ EE Estonia .....
☒ ES Spain .....
☒ FI Finland .....
☒ GB United Kingdom
☒ GD Grenada
☒ GE Georgia .....
☒ GH Ghana .....
☒ GM Gambia
☒ HR Croatia .....
☒ HU Hungary .....
☒ ID Indonesia
☒ IL Israel .....
☒ IN India .....
☒ IS Iceland
☒ JP Japan .....
☒ KE Kenya .....
☒ KG Kyrgyzstan .....
☒ KP Democratic People's Republic of Korea .....
☒ KR Republic of Korea .....
☒ KZ Kazakhstan .....
☒ LC Saint Lucia
☒ LK Sri Lanka
☒ LR Liberia
☒ LS Lesotho .....
☒ LT Lithuania
☒ LU Luxembourg
☒ LV Latvia
☒ MA Morocco .....
☒ MD Republic of Moldova .....
☒ MG Madagascar .....
☒ MK The former Yugoslav Republic of Macedonia .....
☒ MN Mongolia
☒ MW Malawi .....
☒ MX Mexico .....
☒ MZ Mozambique
☒ NO Norway
☒ NZ New Zealand .....
☒ PL Poland .....
☒ PT Portugal .....
☒ RO Romania
☒ RU Russian Federation .....
☒ SD Sudan
☒ SE Sweden
☒ SG Singapore
☒ SI Slovenia .....
☒ SK Slovakia .....
☒ SL Sierra Leone .....
☒ TJ Tajikistan .....
☒ TM Turkmenistan .....
☒ TR Turkey .....
☒ TT Trinidad and Tobago .....
☒ TZ United Republic of Tanzania
☒ UA Ukraine .....
☒ UG Uganda .....
☒ US United States of America continuation-in-part
☒ UZ Uzbekistan .....
☒ VN Viet Nam .....
☒ YU Yugoslavia .....
☒ ZA South Africa .....
☒ ZW Zimbabwe .....

Check-box reserved for designating States which have become party to the PCT after issuance of this sheet:

☐ .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)*

See Notes to the request form

Supplemental Box *If the Supplemental Box is not used, this sheet need not be included in the request.*

*1. If, in any of the Boxes, **the space is insufficient** to furnish all the information: in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:*

*(i) **if more than two persons are involved as applicants and/or inventors** and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;*

*(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication **"the States indicated in the Supplemental Box"** is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;*

*(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, **the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America:** in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;*

*(iv) if, in addition to the agent(s) indicated in Box IV, there are **further agents**: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;*

*(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication **"patent of addition,"** or **"certificate of addition,"** or if, in Box No. V, the name of the United States of America is accompanied by an indication **"continuation"** or **"continuation-in-part"**: in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;*

*(vi) if, in Box No. VI, there are **more than three earlier applications whose priority is claimed**: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;*

*(vii) if, in Box No. VI, **the earlier application is an ARIPO application**: in such case, write "Continuation of Box No. VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which that earlier application was filed.*

*2. If, with regard to the **precautionary designation statement** contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statement: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.*

*3. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning **non-prejudicial disclosures or exceptions to lack of novelty**: in such case, write "Statement concerning non-prejudical disclosures or exceptions to lack of novelty" and furnish that statement below.*

Continuation of Box V:

United States of America, continuation-in-part of USSN 60/155,018 filed 21 September 1999 (21/09/99)

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 21 September 1999 (21/09/99)	60/155,018	US		
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): (1)

** Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):*

ISA/ US

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date *(day/month/year)* Number Country (or regional Office)

Box No. VIII CHECK LIST: LANGUAGE OF FILING

This international application contains the following **number of sheets:**

request	:	6
description (excluding sequence listing part)	:	14
claims	:	2
abstract	:	1
drawings	:	0
sequence listing part of description	:	0
Total number of sheets	:	23

This international application is **accompanied** by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☒ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: Transmittal Letter

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

Jane Massey Licata

LICATA, Jane Massey

Date: 20 September 2000

For receiving Office use only

1. Date of actual receipt of the purported international application:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA/
6. ☐ Transmittal of search copy delayed until search fee is paid.

2. Drawings:
   ☐ received:
   ☐ not received:

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

This sheet is not part of and does not count as a sheet of the international application.

PCT

FEE CALCULATION SHEET
Annex to the Request

For receiving Office use only

International application No.

Date stamp of the receiving Office

Applicant's or agent's file reference: **RU-0103**

Applicant
Rutgers, the State University et al.

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE . . . . . 240.00 T

2. SEARCH FEE . . . . . 700.00 S

International search to be carried out by **ISA/US**

(If two or more International Searching Authorities are competent in relation to the international application, indicate the name of the Authority which is chosen to carry out the international search.)

3. INTERNATIONAL FEE

Basic Fee

The international application contains **23** sheets.

first 30 sheets 427.00 b1

0 remaining sheets x _____ additional amount = 0.00 b2

Add amounts entered at b1 and b2 and enter total at B . . 427.00 B

Designation Fees

The international application contains **82** designations.

8 number of designation fees payable *(maximum 8)* x **92.00** amount of designation fee = 736.00 D

Add amounts entered at B and D and enter total at I . . . . . 1,163.00 I

(Applicants from certain States are entitled to a reduction of 75% of the international fee. Where the applicant is (or all applicants are) so entitled, the

4. FEE FOR PRIORITY DOCUMENT *(if applicable)* . . . . . 15.00 P

5. TOTAL FEES PAYABLE . . . . . 2,118.00 TOTAL

Add amounts entered at T, S, I and P, and enter total in the TOTAL box

☐ The designation fees are not paid at this time.

MODE OF PAYMENT

☒ authorization to charge deposit account (see below)
☐ bank draft
☐ coupons
☐ cheque
☐ cash
☒ other *(specify)*: **Credit Card Payment form**
☐ postal money order
☐ revenue stamps

DEPOSIT ACCOUNT AUTHORIZATION *(this mode of payment may not be available at all receiving Offices)*

The RO/ **US**

☐ is hereby authorized to charge the total fees indicated above to my deposit account.

☒ *(this check-box may be marked only if the conditions for deposit accounts of the receiving Office so permit)* is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

☐ is hereby authorized to charge the fee for preparation and transmittal of the priority document to the International Bureau of WIPO to my deposit account.

Deposit Account No.	Date *(day/month/year)*	Signature
12-1086	20 September 2000	

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

REVISED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 March 2001 (29.03.2001)

PCT

(10) International Publication Number
WO 01/21137 A1

(51) International Patent Classification[7]: A61K 6/00, 7/00, 7/42, 7/44

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(22) International Filing Date:
20 September 2000 (20.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/155,018 21 September 1999 (21.09.1999) US

(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:
US 60/155,018 (CIP)
Filed on 21 September 1999 (21.09.1999)

(71) Applicant *(for all designated States except US)*: **RUTGERS, THE STATE UNIVERSITY** [US/US]; Old Queens Building, Somerset and George Streets, New Brunswick, NJ 08901 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **GHAI, Geeta** [US/US]; 250 Gallinson Drive, Murray Hill, NJ 07974 (US). **ROSEN, Robert, T.** [US/US]; 347 Harrier Drive, Monroe Township, NJ 08831 (US). **HO, Chi-Tang** [US/US]; 32 Jernee Drive, East Brunswick, NJ 08816 (US). **CHEN, Kuang, Yu** [CN/US]; 4 Silverthron Lane, Belle Mead, NJ 08502 (US). **TELANG, Nitin** [IN/US]; 788 Colonial Avenue, Pelham Manor, NY 10803 (US). **LIPKIN, Martin** [US/US]; 535 East 86th Street, New York, NY 10028 (US). **HUANG, Mou, Tuan** [US/US]; 266 Alfred Street, Englewood Cliffs, NJ 07632 (US). **BOYD, Charles** [US/US]; 3330 Paty Drive, Honolulu, HI 96822 (US). **CSISZAR, Katalin** [HU/US]; 3330Paty Drive, Honolulu, HI 96822 (US).

(74) Agents: **LICATA, Jane, Massey** et al.; Law Offices of Jane Massey Licata, 66 E. Main Street, Marlton, NJ 08053 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the revised international search report: 26 July 2001

(15) Information about Correction:
see PCT Gazette No. 30/2001 of 26 July 2001, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/21137 A1

(54) Title: EXTRACTS OF ORANGE PEEL FOR PREVENTION AND TREATMENT OF CANCER

(57) Abstract: Compositions and methods of inhibiting tumor cell growth and treating and preventing cancer are provided based on administration of an orange peel extract either alone or in combination with other phytochemicals.

REVISED VERSION

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/25733

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 6/00, 7/00, 7/ 42, 7/44
US CL : 424/59.60, 63, 69, 195.1, 400,,401, 448; 426/425, 428; 435/ 209, 267; 514/733,736, 844, 846,847, 887

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 424/59.60, 63, 69, 195.1, 400,,401, 448; 426/425, 428; 435/ 209, 267; 514/733,736, 844, 846,847, 887

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Please See Continuation Sheet

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NAGY, S. et al. Citrus Science and Technology.Westport:AVI. 1977, Vol.1, page 415, lines 40-42; Page 416, lines 15-41, pages 415-419	1,2
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Y		3-8
X	PEIRCE, Andrea. Practical Guide to Natural Medicines. New York, William Morrow and Company. 1999, pages 551-554, especially page 553, lines 5-7.	1,3,5,7
X	Madis Botanicals, Inc. ResveraPure™ Resveratrol PE 8%, lines 6-7 and 15-31	1-2,3,5,7,8
X	CASTLEMAN. Michael. The Healing Herbs. Emmaus: Rodale Press. 1991, pages 348-350, especially page 349, column 2, lines 5-10.	1,3,5,7
Y	US 5, 830, 738 A (THOMAS et al.) 03 November 1998 (03.11.98),Col. 1, lines 22-62.	1-4
Y	US 5,859, 293 A (BAILEY et al.) 12 January 1999, (12.01.1999), column 1, lines29-34; Column 2, lines 10-15.	3,4,6-8

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
06 November 2000	27 DEC 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Kailash C. Srivastava for Telephone No. (703)-308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

Continuation of B. FIELDS SEARCHED Item 2: PEIRCE, A. (Ed.) Practical Guide to Natural Medicines. New York. Wiliam Morrow and Company, Inc.,1999, Pages 551-554.
NAGY, S., SHAW, P.E., VELDHUIS, M.K. (Eds.) Citrus Science and Tcehnology. Westport: AVI Publishing, Co., Inc., 1979, Vol.1, pages 415-419; Page 415, Lines 40-42; Page 416, Lines15-41.
CASTLEMAN, M. The Healing Herrbs. Emmaus: Rodale Press,1991, Pages 348-350, especially page 349,Column 2, lines5-10

Continuation of B. FIELDS SEARCHED Item 3: CAS, USPT, JPAB, EPAB, DWPlorange peel extarct, japanese knotwood, Polygonum cuspidatum, huzhang, mexican bamboo, hydroxyflavone, hexamethoxyflavone, rosemary, blacktea, hazhang extract, resveratrol analog, cancer treatment, tumor prevention,sinensetin, nobeeltin, tangeretin, auranetin)